Clinico-experimental Studies on the Significance of the Forms of the Polarographic Protein Double Wave. (I) : Changes of the Filtrate Wave (Brdicka) by Means of Serum Denaturation

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NOTE

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Changes of the Filtrate Wave (Brdicka) by Means of Serum Denaturation

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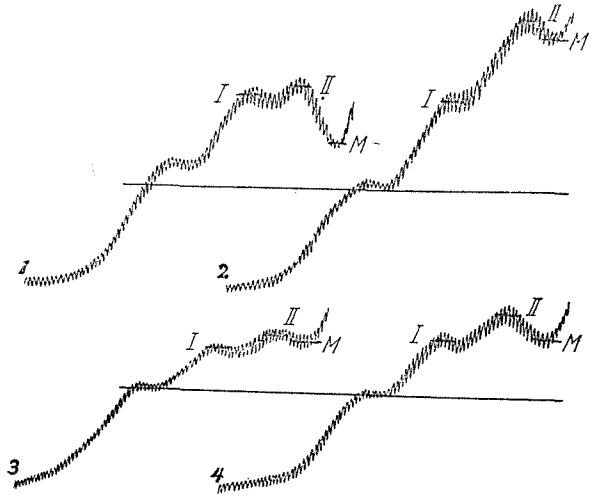
The forms of protein double waves mainly consist of first and second maximums. They are very variable, depending upon such many factors as protein structure, concentration and denaturation and the characters of buffer solutions containing cobaltous or cobaltic chloride and so on. If the definite conditions are given, however, the definite protein wave can be obtained. For instance, the protein wave of serum albumin evidently differs from that of serum globulin in respect to the wave height or to the wave from (Fig. 1).



1, 3 : native 2, 4 : denatured states

Fig. 1. Protein wave of serum albumin (1, 2) and serum globulin (3, 4) from cobaltous buffer solution.

What is the meaning of each of this double waves? Recently findings[1] have been accumulated that the second maximum of protein double wave could be attributed to the thiol or disulfide groups in the protein molecule.

Nevertheless, with regard to the origin of first maximum, there are no reasonable hypothesis at present. We have tried to clear up this problem

from the clinical view-point. In our investigations of the filtrate test on about 200 samples, special attention was paid to the changes of filtrate wave occuring by alkaline denaturation. For this purpose, on each serum-sample, the sulfosalicylic (SSA) filtrates were examined both in the native and in the denatured states, e. g. before and after the addition of KOH solution. The conditions of denaturation, of course, were kept strictly the same.

The results showed that the transformation of double wave by alkaline action occurred always towards the same direction, namely from the left to the right in Fig. 2. Therefore, it can be said that in consequence of the advance of the serum denaturation the first maximum of filtrate wave becomes gradually lower, while the second higher.

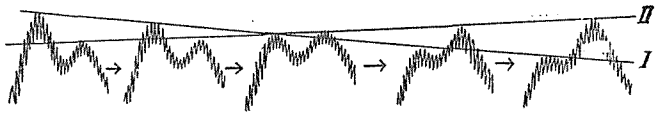


Fig. 2. Changes of Wave from (I, II) in the SSA filtrates by the denaturation of serum.

Morever, it became evident that these changes of wave forms had no relationship to the phenomenon of "Kreuzungeffekt"[2], because of the independency of the changes of wave forms on their protein concentrations.

After the confirmation that the same changes took place also in the heat denaturation, the following experiment was carried out. The mixture of serum and SSA were at first heated to the definite extent; and then filtered. By addition of phosphotungstic acid to this filtrate, the mucoprotein was obtained as the precipitate[3]. Using this mucoprotein solution, the contents of nitrogen, polysaccharide and polarographic protein wave were examined. The result (Table 1) showed that, in spite of the existence of parallelism between the wave heights of the second maximum and the nitrogen concentration, the polysaccharide portion of mucoprotein always decreased

Table 1. Changes of the mucoprotein of the serum when heated with SSA.

Condition of heating	Polysaccharide ** mg/dl (filtrate)	Nitrogen* mg/dl	P/N	Protein Wave Height (II) mm, S : 1/50
control	18.5 (75.0)	14.58	1.27	97
90°C, 5 minutes	9.5 (80.0)	13.42	0.71	72
〃 ,10 〃	10.0 (85.0)	12.25	0.82	87
〃 ,20 〃	16.7 (90.0)	19.25	0.87	124
〃 ,30 〃	14.0 (90.0)	20.22	0.70	124

* Kjeldahlometry. ** Orcinol test.

(contrary to that of the filtrate which is free from mucoprotein) in agreement with the decrease of first maximum.

Thus, we reached to the assumption that the decrease of the first wave by the denaturation of serum-sample may be attributed to the decrease of polysaccharide of the mucoprotein.

Most recently, E. Balle-Helaers[4] found an important fact that, if the tylose (polysaccharide) is added to the solution of cystamine, which reveals only the second maximum, the typical double wave can be obtained. In consideration of this new finding, our clinico-experimental data seems quite interesting.

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